measuring CDK4 kinase activity of the cell, wherein a test compound which inhibits said CDK4 kinase activity is identified as a candidate agent with anti-cancer activity.

REMARKS

Amendments

Claim 25 has been amended to recite that "the genetic alteration causes *c-MYC* overexpression." This amendment is supported by the specification which discloses that dysregulation of c-MYC includes "overexpression of c-*MYC* [that] can be traced to genetic alterations of the oncogene itself, . . . [or] genetic defects in upstream regulators of *c-MYC* expression." (Page 1, lines7-9.)

Claim 25 has also been amended to recite measuring "the CDK4 kinase activity of the cell" in place of measuring "activity of CDK4 in the cell." This amendment is supported by the specification which discloses, "Test compounds can be contacted with such cells and their effects on the cells' CDK4 enzymatic activity can be monitored." (Page 8, lines 26-28.) The amendment is also supported by the specification which discloses, "Methods for assaying for CDK4 enzymatic (kinase) activity are known in the art." (Page 8, line 29 to page 9, line 1.)

These amendments introduce no new matter.

The Rejection of Claims 25-32 Under 35 U.S.C. § 112, Second Paragraph

Claims 25-32 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite. Applicants respectfully traverse. The Office Action asserts three separate reasons as to why the claims are indefinite. Each will be discussed in turn.

1. The Office Action asserts that claims 25-32 are indefinite for reciting "a genetic alteration which dysregulates *c-MYC* expression" because the specification discloses that an

alteration which dysregulates *c-MYC* expression includes overexpression of *c-MYC*, but not other types of dysregulation, such as under-expression of *c-MYC*. (Paper 13, page 3, lines 13-17.) Applicants do not agree with the Patent Office's characterization of the specification; nonetheless, to advance prosecution, the claims have been amended to recite "wherein the genetic alteration causes *c-MYC* overexpression."

2. The Office Action asserts that claims 25-32 are indefinite for reciting "activity of CDK4" because it is unclear what activities of CDK4 can be measured. (Paper 13, page 3, lines 22-25.)

To advance prosecution, claim 25 has been amended recite that "CDK4 kinase activity" is measured in a cell. One of skill in the art would understand the CDK4 kinase activity is an enzymatic activity in which CDK4 phosphorylates a substrate protein. Substrate proteins of CDK4 were also known in the art at the time the application was filed. The specification discloses that "a target of CDK4 phosphorylation is the retinoblastoma tumor suppressor gene product pRB." (Page 20, lines 19-20.) Other substrates of CDK4 phosphorylation known in the art included histone H1. Thus one of skill in the art would have readily understood the scope of claims 25-32 as originally filed, but the amendment clarifies the intended meaning.

3. The Office Action asserts that claims 25-32 are indefinite because they recite a step of measuring CDK4 activity, but it is unclear how to do so because the specification does not explicitly teach an assay that directly measures CDK4 activity. (Paper 13, page 4, lines 1-4.)

The claims of a patent application must meet two requirements under 35 U.S.C. § 112, second paragraph:

> (A) the claims must set forth the subject matter that applicants regard as their invention; and
>
> (B) the claims must particularly point out and distinctly define the metes and bounds of the subject matter that will be protected by the patent grant.

MPEP § 2171.

First, the claim recitation "measuring activity of CDK4" has been amended to "measuring CDK4 kinase activity." This recitation sets forth the subject matter which applicants regard as their invention (requirement (A)). The invention set forth by the claims is presumed, in the absence of evidence to the contrary, to be that which applicants regard as their invention. *In re Moore*, 439 F.2d 1232 (CCPA 1971). Thus the claims meet prong (A) of 35 U.S.C. § 112, second paragraph.

Second, the claim recitation "measuring CDK4 kinase activity" distinctly defines the meets and bounds of the claimed subject matter (requirement (B)). This requirement is to ensure that the scope of the claims is clear so that the public is informed of the boundaries of what constitutes infringement of the patent. See MPEP § 2173. CDK4 kinase activity is CDK4's ability to phosphorylate substrate proteins. Thus the claims meet prong (B). Thus the claims fulfill both prongs set forth in the MPEP.

The rejection fails to assert any reason, consistent with the requirements under 35 U.S.C. § 112 second paragraph, why "measuring CDK4 kinase activity" is unclear. Rather, the Office Action asserts that it is unclear how to measure CDK4 activity using the specification as a guide. This assertion appears to concern the enablement requirement of 35 U.S.C. § 112, first paragraph.[1] Enablement, however, is not a requirement of 35 U.S.C. § 112, second paragraph.

Withdrawal of these rejections to claims 25-32 is respectfully requested.

[1] This concern is addressed below.

The Rejection of Claims 25-32 under 35 U.S.C. § 112, first paragraph

Claims 25-32 are rejected under 35 U.S.C. § 112, first paragraph, as allegedly not enabled by the specification. The rejection asserts that one of skill in the art would not know how to make and/or use the invention from the specification. The rejection is respectfully traversed.

Claim 25 is the independent claim of the rejected claim set. Claim 25 recites a method of screening compounds to identify as candidates those which have anti-cancer activity. A cell that has a genetic alteration which dysregulates *c-MYC* expression is contacted with a test compound. The genetic alteration causes *c-MYC* overexpression. The CDK4 kinase activity of the cell is measured. A test compound which inhibits CDK4 kinase activity is identified as a candidate agent with anti-cancer activity.

To satisfy the enablement requirement, the specification of a patent must teach those skilled in the art how to make and use the full scope of the claimed invention without undue experimentation. *In re Wright*, 999 F.2d 1557 (Fed. Cir. 1993). A patent need not teach, and preferably omits, what is well known in the art. *In re Buchner*, 929 F.2d 660, 661 (Fed. Cir. 1991); *Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1384 (Fed. Cir. 1986).

The Office Action asserts that the claims are not enabled because undue experimentation would be required to determine how to measure CDK4 kinase activity directly from cells contacted with an agent. The Office Action asserts, "The specification does not teach how to measure the CDK4 kinase activity directly from the agent contacted cells . . . and the art seems to teach CDK4 kinase activity is measured in vitro." (Paper 13, page 4, lines 11-21.) The Office Action seems to have construed the claim as filed to require testing *in vivo* or in whole cells. This, however, was not the intention. Claim 25 has been amended to clarify any ambiguity.

Amended claim 25 recites that "CDK4 kinase activity of the cell" is measured. This step of amended claim 25 is supported by the specification such that one of skill in the art would understand how to measure a cell's CDK4 kinase activity. The specification discloses, "Methods for assaying for CDK4 enzymatic (kinase) activity are known in the art and any such method can be used." (Page 8, line 29 to page 9, line 1.) Indeed, methods of measuring a CDK4 kinase activity of a cell were well known in the art prior to the February 11, 2000 effective filing date of the application. See Exhibits A-H below.

Fukuchi *et al.* (*Biochimica et Biophysica Acta* (1997) *1357*, 297-305; Exhibit A) discloses the measurement of CDK4 kinase activity by determining the phosphorylation status of pRB. "To investigate CDK4 function, we examined the phosphorylation status of pRB." (Page 301, column 2, lines 22-23.) Phosphorlyation of pRB was performed using an enzyme-linked immunosorbent assay (ELISA). Beads coated with anti-pRB antibodies were incubated with cell lysates. The beads were then incubated with rabbit anti-phosphorylated serine and threonine polyclonal antibodies followed by anti-rabbit antibodies conjugated to horse peroxidase. Phosphorylated pRB was detecting by adding ECL [enhanced chemiluminescence] solution and measuring chemiluminescence. (Page 299, column 1 lines 3-31.)

Kulp *et al.* (*Experimental Cell Research* (1996) *229*, 60-68; Exhibit B) measured the CDK4 kinase activity of cells treated with iron chelators by determining the phosphorylation status of Rb (pRB) in cells. "As a potential consequence of the decreased protein levels of the cyclin D/cdk4 complex, we examined the state of phosphorylation of Rb (as evidenced by altered migration on SDS gels) by Western blotting. Retinoblastoma protein is a physiological substrate of cyclin D and cdk4." (Page 63, column 2, line 10 to page 64, column 1, line 1.)

Phelps *et al.* (*Methods in Enzymology* (1997) *283*, 194-205; Exhibit C) provides a protocol for measuring cellular CDK4 kinase activity, called an immunoprecipitation (IP)-kinase assay. Phelps provides detailed instructions for performing each step of the IP-kinase assay. These steps include lysing mammalian cells (see "*Lysis of Mammalian Cells*" at page 197, line 9 to page 198, line 20), immunoprecipitating CDK4 from the lysed cells (see "*Immunoprecipitation* at page 198, line 21 to page 199, line 16), and assaying the enzymatic activity of the immunoprecipitated CDK4 (see "*CDK4 and CDK6 Kinase Assay*" at page 204, lines 1-34). Phelps teaches that enzymatic (kinase) activity of CDK4 is assayed by incubating CDK4 with at GST-pRB fusion protein, [γ-^{32}P]ATP, and kinase reaction buffer. The reaction products are run on an SDS-PAGE gel and exposed to a phosphorimager plate or X-ray film to detect transfer of the labeled phosphate to pRB. Phelps also teaches that these assays are specific and sensitive at detecting *in vivo* CDK4 kinase activity. "The assay of kinase activity by immunoprecipitation (IP-kinase assay) provides not only a specific and sensitive, but sometimes the only, measurement for studying the regulation of a particular growth condition (e.g., differentiation, senescence, negative and positive cytokines) on the activity of a CDK *in vivo*." (Page 196, lines 12-16.)

Li *et al.* (*Experimental Cell Research* (1999) *253*, 372-384; Exhibit D) quantitated cellular Cdk4 kinase activity as follows: "Equivalent amounts of protein extract . . . were then added to protein A/protein G agarose beads (Santa Cruz) that had been precoated with 2 μg polyclonal Cdk4 . . . antibodies. . . . Complexes were immunoprecipitated . . . Immunoprecipitates were washed and then resuspended in 30 μl kinase reaction mix . . . containing 2 mg bacterially expressed GST-Rb . . . as substrate. After incubation at 30°C for 30 min, the reactions were stopped by the addition of SDS sample buffer and then boiled for 5 min,

electrophoresed through 7.5% SDS-PAGE, and exposed to X-ray film. The amount of kinase activity was quantified by use of a phosphorimager." (Page 374, column 1, lines 24-41.)

Zhou *et al.* (*Breast Cancer Research and Treatment* (2000) *59*, 27-39; Exhibit E) measured Cdk4 activity in "[e]xponential cultures of MCF-10A control or cyclin D1 transfectants [that] were lysed in lysis buffer. . . . Five hundred microgram lysates were precleared with protein A-sepharose before the supernatants were incubated with 5 μg cyclin D1 antibody . . . The immune complexes were precipitated by protein A-sepharose and cyclin D1 kinase activity was determined using 0.45 μg GST-Rb (Santa Cruz) and 10 μCi [γ-^{32}P]ATP (ICN) as described previously [37]." (Page 29, column 2, lines 10-24.)

Krynska *et al.* (*Journal of Cellular Biochemistry* (1997) *67*, 223-230; Exhibit F) also discloses an assay for Cdk4 activity. A "protein extract from normal and transgenic tissues was immunoprecipitated overnight at 4°C with specific antibodies . . . The immunoprecipitates were assayed for kinase activity for 20 min in reaction buffer containing . . . 5 μCi [γ ^{32}P]-ATP, and 200 mg/ml calf thymus histone H1 . . . in a final volume of 50 μl. The reaction was carried out at 30°C for 20 min and stopped by the addition of 50 μl of sample loading buffer. Phosphorylated histone H1 was identified following SDS-PAGE and autoradiography." (Page 224, column 2, line 51 to page 225, column 2, lines 16.)

Jamout *et al.* (*Hepatology* (1999) *29*, 385-395; Exhibit G) measured the enzymatic activity of cdk4 in liver cells by the IP-kinase assay. "To determine cdk4 activity, samples were lysed in buffer G . . . IPs [immunoprecipitations] were performed . . . The kinase assays were performed as described by Matsushime et al. . . . Cdk4 immunoprecipitates were incubated at 30°C for 30 min in kinase buffer . . . containing 5 mg of soluble GST-pRb fusion protein . . . and 10 μCi of γ-^{32}P-ATP . . . in a total volume of 25 μL. Reaction was stopped by adding Laemmli

sample buffer, and then the samples were electrophoresed in 10% sodium dodecyl sulfate-polyacrylamide gels. The gels were dried and the phosphorylated proteins were visualized by autoradiography." (Page 387, column 1, lines 18-39.)

Beuchamp *et al.* (*Annals of Surgery* (1996) *223*, 620-628; Exhibit H) assayed Cdk4 activity of growth-arrested cells: "The cell were dispersed into IP buffer . . . The protein concentrations of each lysate then were determined, and equivalent amounts of protein were subjected to kinase assay . . . The Rb kinase assay then was performed for 30 minutes at 30°C in Rb kinase buffer . . . with the addition of 0.1 μg GST-Rb, 20 μM cold ATP, and 10 μCi [γ-^{32}P]ATP in a final volume of 30 μL. The reactions were stopped with the addition of 30 mL 2x Laemmli buffer and then boiling for 5 minutes, then were analyzed by SDS-PAGE and autoradiography." (Page 623, column 1, lines 6-31.)

The Exhibits clearly demonstrate that assays used to measure CDK4 kinase activity of a cell were well known at the time the application was filed. Thus, one of skill in the art could have readily performed the step of "measuring CDK4 kinase activity of the cell" as recited in claims 25-32 without having to resort to undue experimentation. Thus the specification clearly enables one of skill in the art to practice the invention.

Withdrawal of this rejection to claims 25-32 is respectfully requested.

The Rejection of Claims 25-32 Under 35 U.S.C. § 102(a)

Claims 25-32 are rejected under 35 U.S.C. § 102(a) as anticipated by Kubo *et al*, (*Clinical Cancer Research* (1999) *5*, 4279-4286; "Kubo").

"A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631 (Fed. Cir. 1987). To establish inherency of the

claimed invention, extrinsic evidence "must make clear that the missing descriptive matter is necessarily present in the thing described in the reference, and that it would be so recognized by persons of ordinary skill." *Continental Can Co. v. Monsanto Co.*, 948 F.2d 1264, 1268 (Fed. Cir. 1991). Thus, "[i]n relying upon the theory of inherency, the examiner must provide a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent characteristic necessarily flows from the teachings of the applied prior art." *Ex parte Levy*, 17 USPQ2d 1461, 1464) (Bd. Pat. App. & Inter. 1990) (emphasis in original). "When the PTO shows a sound basis for believing that the products of the applicant and the prior art are the same, the applicant has the burden of showing they are not." *In re Spada*, 911 F.2d 705, 709 (Fed. Cir. 1990).

Claim 25 is the independent claim of the rejected claim set. The method of claim 25 comprises two steps: "contacting a cell which has a genetic alteration which dysregulates *c-MYC* expression with a test compound" and "measuring CDK4 kinase activity of the cell." Kubo does not expressly or inherently teach either of these steps.

Kubo "identified specific small molecule inhibitors of CDK4 by comparing the growth-inhibitory activity of more than 50,000 compounds with the p16 status of the cell lines in the NCI drug screen panel." (Page 4285, lines 18-21.) To make this determination, Kubo characterized the p16 gene in each of 60 cancer cell lines that had previously been contacted with each of 50,000 different compounds on that cell line. The status of the p16 gene in each cell line was compared to the growth-inhibitory activity of each of the compounds. Compounds that preferentially inhibited the growth of cancer cell lines with p16 gene mutations were identified and tested for inhibitory activity on purified CDK4. Kubo determined that an agent that

preferentially inhibits the growth of p16-altered cancer cell lines is likely to be an inhibitor of CDK4.

Kubo does not expressly or inherently teach the first step of the claimed method, *i.e.*, "contacting a cell which has a genetic alteration which dysregulates *c-MYC* expression with a test compound." Kubo teaches that 60 different human cancer cell lines had been contacted with each of 50,000 different compounds. (Page 4280, column 1, lines 25-27.) There is no suggestion in Kubo that these 60 cell lines are dysregulated for c-MYC expression. The Patent Office nonetheless alleges inherency: "Limitations of claims 26-32 as well as the limitation in claim 25 'dysregulation of c-MYC expression' appear to be inherent properties of the 60 NCI drug screening panel cancer cells." (Paper 13 page 5, lines 14-17.) The Patent Office offers no evidence or sound scientific reasoning to support its position that one or more of the 60 NCI cell lines are dysregulated for c-MYC. The Patent Office erroneously asserts:

> In the absence of evidence to the contrary, the burden is on the applicant to prove that the 60 different human cancer cell lines NCI selected for drug screening do not have characteristics, i.e. dysregrulates [sic] c-myc, Burkitt's Lymphoma, neuroblastoma, colon cancer, t8:14 translocation, a genetic amplication [sic]of c-myc, a mutation in APC, a truncating mutation in APC.

Paper 13, page 5, lines 22-26. See *Ex parte Levy*, *supra*, which puts the burden of proving inherency squarely on the Patent Office.

The Patent Office has failed to shift the burden to applicants to provide evidence that Kubo does not teach a cell line with a *c-MYC* gene whose expression is dysregulated, because the Patent Office has provided no basis in fact and/or technical reasoning to reasonably support its position. Because the Patent Office has shown absolutely no basis for believing that the cell

lines taught by Kubo have a genetic defect that results in c-MYC dysregulation, applicants are not required to prove otherwise.

Kubo teaches only three characteristics of the cell lines used in his study: that they are "human cancer cell lines" (page 4280, column 1, line 27), that the growth of some of them is inhibited by some of the 50,000 compounds (page 4280, column 1, lines 11-14) and that some of them carry mutations in p16 (see Table 1, page 4281).[2] None of these characteristics, alone or combined, leads to the inevitable conclusion that the cell lines have a genetic alteration that causes dysregulation of c-MYC expression.

Kubo also does not expressly or inherently teach the second step of the claimed method, *i.e.*, "measuring CDK4 kinase activity of the cell." "The cell" refers to the c-MYC dysregulated cell of the first step which was contacted with a test compound. Kubo teaches testing CDK4 made in Sf9 cells. "These compounds were assessed for CDK4:cyclin D kinase inhibitory activity using crude Sf9 insect cell lysate containing baculovirus-expressed CDK4:cyclin D1 and a GST-Rb fusion protein as substrate." (Page 4282, column 1, lines 7-10.) The Sf9 cells of Kubo are not one of the panel of human cancer cell lines. (Sf9 cells are insect cells as stated by Kubo.) Kubo does not teach that the Sf9 cells had been contacted with the test compounds prior to making the lysates which were tested for inhibitory activity. Thus Kubo does not expressly or inherently teach measuring CDK4 kinase activity of the cell, *i.e.*, the cell that had been contacted with a test compound, as required in claim 25.

Kubo does not expressly or inherently teach either "contacting a cell which has a genetic alteration which dysregulates c-MYC expression" or "measuring CDK4 kinase activity of the

[2] p16 and c-MYC are not the same protein and are not encoded by the same gene. The p16 protein is encoded by a 567-nucleotide sequence over three exons of chromosome 9. The p16 protein has 189 amino acid residues. See Exhibit I. The c-MYC protein is encoded by a 4053-nucleotide sequence over two exons on chromosome 8. The c-MYC protein has a molecular weight of 48,812 D (approximately 439 amino acid residues). See Exhibit J.

cell" as recited in claim 25. Claims 26-32 depend from claim 25 and thus also contain these recitations. Therefore, none of these claims are anticipated.

Withdrawal of this rejection to claims 25-32 is respectfully requested.

Objection of the Specification

The specification has been objected to for improper incorporation of essential material by reference to a foreign application or patent, or to a publication. Specifically, the Office Action asserts that material essential to the practice of the invention, *i.e.*, how to measure "CDK4 activity," is disclosed in the specification by reference to a publication, Li *et al.* (*Biochemistry* (2000) *649*, 647-657), which is impermissible." (Paper 13, page 2, lines 17-22.) Applicants respectfully traverse that this subject matter is essential.

As discussed above with regard to the enablement requirement of 35 U.S.C. § 112, first paragraph, CDK4 kinase assays were well known in the art at the time the application was filed. Since those of skill in the art could have readily found such assays in the literature, the Li reference is not essential to the practice of the invention.

Thus the citation of Li is not improper because it is not essential.

Withdrawal of this objection to the specification is respectfully requested.

Respectfully submitted,

Date: Jan. 21, 2003

By: Michelle Holmes-Son
Michelle Holmes-Son
Registration No. 47,660

Appendix I. Marked up version of the claims to show the changes made

25. (Twice Amended) A method of screening compounds to identify as candidate agents those which have anti-cancer activity, comprising the steps of:

contacting a cell which has a genetic alteration which dysregulates *c-MYC* expression with a test compound, wherein the genetic alteration causes *c-MYC* overexpression;

measuring [activity of] CDK4 kinase activity [in] of the cell, wherein a test compound which inhibits [activity of] said CDK4 kinase activity is identified as a candidate agent with anti-cancer activity.